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Development, seasonal polyphenism and cold hardiness of the blue pansy, *Junonia orithya orithya* (Lepidoptera, Nymphalidae)

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Abstract The blue pansy, *Junonia orithya orithya* (Lepidoptera, Nymphalidae), was reared under a photoperiod of 12L–12D (short day) at 20 or 25°C or under 16L–8D (long day) at 20, 25 or 30°C, and larval development, seasonal polyphenism and cold hardiness were examined. The mean duration of larval and pupal stages decreased significantly with increase in temperature. The developmental threshold (t_0) and the thermal constant (K) for the larval and pupal stages were 13.7°C and 208.3 day-degrees, and 13.4°C and 99 day-degrees, respectively. Although all the adult females maintained under 16L–8D at 20 and 25°C produced mature eggs one week after emergence, none of those under 12L–12D at 20°C had produced mature eggs even 14 days after adult eclosion. Adults exhibited seasonal polyphenism in response to the photoperiod and temperature experienced at the larval and pupal stages. Under long-day photoperiods at high temperatures, eyespots developed on the ventral surface of the hind wing in both sexes, with an orange pattern also appearing on the dorsal surface of the hind wing in females only (long-day form). In contrast, under short day at low temperature, the eye spots were obscure and the dorsal surface of the hind wing was usually blue in females (short-day form). The supercooling points for larvae, pupae and adults were approximately −12, −17 and −20°C, respectively, irrespective of photoperiodic conditions.

Key words Development, seasonal polyphenism, cold hardiness, distribution, *Junonia orithya orithya*, butterfly, Japan.

Introduction

The blue pansy, *Junonia* (=*Precis*) *orithya orithya* (Lepidoptera, Nymphalidae), is distributed in China, Taiwan and Japan (*e.g.* Shirôzu, 1960; Tsukada, 1985; Igarashi and Fukuda, 1997). While little is known about the overwintering of this species in mainland Japan, the adults are known to overwinter in southwestern Okinawa, Japan (Uesugi, 1998; Shirôzu, 2006). The occurrence of this species in Honshu, Shikoku and Kyushu is likely due to the annual northward dispersal of adults from southern Japan, and records have increased in recent years throughout western Japan (Nakagami, 1997; Yoshio, 2002; Shirôzu, 2006, Fukuda 2004, 2008). This species is known to exhibit marked seasonal polyphenism of wing pattern in the field (*e.g.* Nobayashi, 2002). At temperate latitudes, photoperiod is the primary cue affecting the adult phenotype of polyphenic butterflies, with temperature also known to be involved (*e.g.* Danilevskii, 1961; Shapiro, 1976; Kato, 1986; Masaki and Yata, 1988). Although Nakanishi *et al.* (1975) investigated seasonal polymorphism in this species under different photoperiods at 25°C, the combined effect of photoperiod and temperature remained unclear.

In the present study, effects of photoperiod and temperature on seasonal polyphenism of adults in this species were examined under laboratory conditions. The supercooling point (SCP), which is generally regarded as the lower limit of survival and used as a convenient measure of insect cold-hardiness (*e.g.* Sømme, 1982), was also measured to elucidate the factors involved in the recent northward expansion of this species.

Materials and methods

Eggs of *J. o. orithya* were obtained from a female captured on Iriomote Island, Okinawa Prefecture, southwestern Japan (*c.a.* 24°N, 123°E) in July 2004. In order to determine the effects of photoperiod and temperature on wing pattern and ovarian development in adults, larvae, pupae and adult females were reared under a photoperiod of 12L–12D (short day) or 16L–8D (long day) at temperatures of 20, 25 or 30°C. The larvae were reared on a mixture of artificial diet (Insecta F-II, Nihon-Nosan-Kogyo Co., Japan) and 30% dried leaf powder prepared from the host plant, water willow, *Justicia procumbens* (see Hirai and Ishii, 2001). The 1st to 4th instars were kept in clear plastic Petri dishes (diameter: 5 cm, depth: 1 cm) and the 5th (final) instars were kept in 200 ml clear plastic cups. Under the same conditions as those used to maintain larvae, adult females were kept individually in triangular glassine envelopes and fed 10% sugar water *ad libitum* every day for 0–14 days after adult emergence. The abdomens of females were dissected under a stereomicroscope 0, 3, 7 or 14 days after adult emergence and the number of mature eggs in the ovary was recorded. Eggs with a height of approximately 0.5 mm and with longitudinal ribs on the chorion

Fig. 1. Variation of number of eyespots (arrows) on a ventral hindwing in *J. orithya orithya*. a: no spot, b: single spot, c: two spots.

Fig. 2. Color pattern variation of dorsal hind wing in female *J. orithya orithya*. a: blue, b: blue and orange, c: orange.

Table 1. Mean larval and pupal stages (days ± SE) of *J. orithya orithya* under different temperature and photoperiodic conditions

Condition		No. of larval instars before pupation	Larva	*N*	Pupa	*N*
30°C	16L–8D	5	12.5 ± 0.12a	33	6.1 ± 0.07a	32
25°C	16L–8D	5	19.7 ± 0.52c	13	8.4 ± 0.23b	12
		6	20.2 ± 0.25c	18	8.3 ± 0.14b	18
	12L–12D	5	17.4 ± 0.25b	15	9.0 ± 0.15b	14
		6	18.8 ± 0.21bc	22	9.0 ± 0.11b	22
20°C	16L–8D	5	30.3 ± 0.33d	23	15.7 ± 0.13c	22
		6	33.3 ± 0.75e	4	16.5 ± 0.29c	4
	12L–12D	5	34.5 ± 0.45e	10	18.7 ± 0.30d	10
		6	36.6 ± 0.33f	17	18.6 ± 0.23d	14

Means followed by the same letter in each column are not significantly different at 5% levels by Tukey-Kramer HSD test.

were considered to be mature eggs, and females having at least one mature egg in the ovary were regarded as mature. Based on the relationship between temperature and developmental rate, the developmental thresholds (t_0) and thermal constants (K) for larval and pupal stages were calculated. In addition, the number of eyespots on the ventral hindwing in both sexes (0, 1 or 2; Fig. 1), and the color of the dorsal hind wing in females ('blue', 'blue & orange' or 'orange'; Fig. 2) were also recorded. The SCPs of the 4th instar larvae, pupae and adults were also measured using a method of Yoshio and Ishii (2001) slightly modified. Prior to the SCP measurements, individual insects were acclimated under short-day at 15°C for at least 3 days.

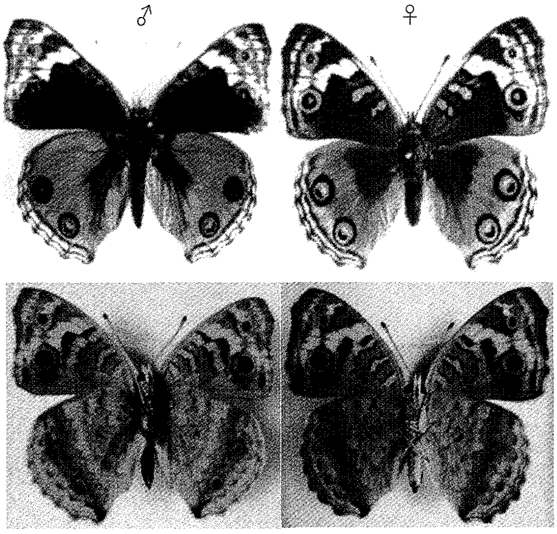
Results

Under the long day at 30°C, all individuals passed through five larval instars before pupating. At 20 and 25°C, however, larvae passed through five or six instars both under the long- and short-day conditions (Table 1). Mean duration of larval and pupal stages decreased significantly with increase in temperature. At 20°C, the mean duration of larval and pupal stages was significantly shorter under long-day conditions than under short-day conditions for both types of individuals which pupated after 5 and 6 instars. The t_0 and K for the larval and pupal stages was 13.7°C and 208.3 day-degrees, and 13.4°C and 99 day-degrees, respectively (Table 2).

Table 2. The regression lines for temperature (t) and developmental rate (V), develpmental threshold (t_0) and thermal constants (K) calculated by relations between temperatures and developmental rates of eggs and pupa of *J. orithya orithya*

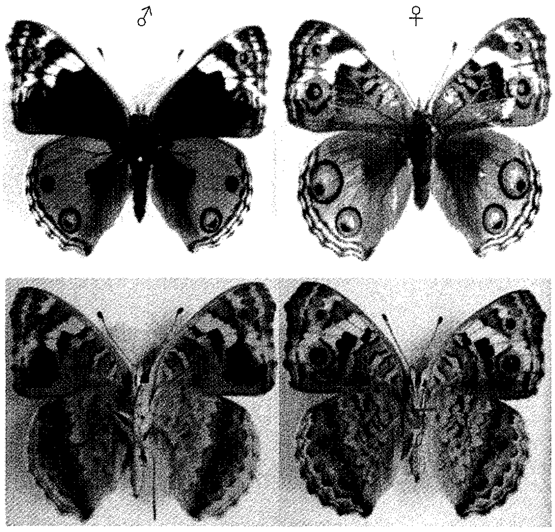
Stage	Regression line	r^2	t_0 (°C)	K (day-degrees)	*N*
Larva	$V = -0.066 + 0.0048t$	0.95***	13.7	208.3	88
Pupa	$V = -0.14 + 0.010t$	0.96***	13.4	99.0	91

***: significance at 0.1 % level by *t*-test

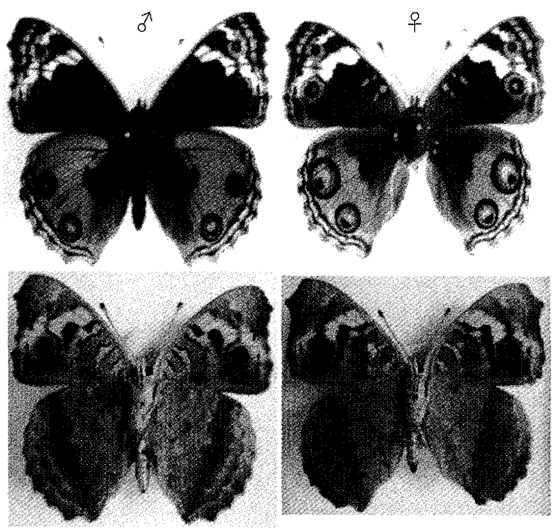


30°C16L–8D

Fig. 3. Male and female adults of *J. orithya orithya* reared under different photoperiod and temprature conditions.

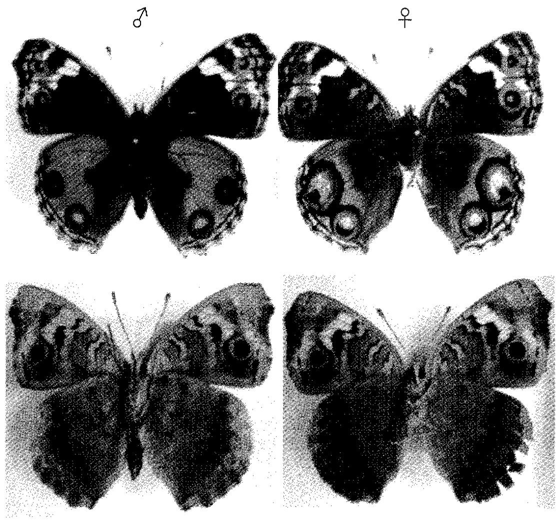


25°C16L–8D



25°C12L–12D

20°C16L–8D



20°C12L–12D

Table 3. Percentages of females with mature eggs 0, 3, 7 and 14 days after adult emergence. Sample sizes are shown in parentheses.

Condition		Days after adult emergence 0	3	7	14
25°C	16L-8D	0 (1)	20.0 (5)	100 (2)	–
	12L-12D	0 (1)	33.3 (6)	16.7 (6)	–
20°C	16L-8D	0 (1)	0 (6)	100 (2)	–
	12L-12D	0 (1)	0 (4)	0 (4)	0 (2)

Table 4. Numbers (%) of *J. orithya orithya* individuals with 0, 1or 2 eye spots on a ventral hindwing, and females with wing color pattern of Blue, Blue & Orange and Orange

Condition		Number of eyespots on a ventral hindwing: Male			Female			Wing pattern of females		
		0	1	2	0	1	2	Blue	Blue & Orange	Orange
30°C	16L–8D	0 (0)	4 (30.8)	9 (69.2)	0 (0)	2 (13.3)	13 (86.7)	1 (6.7)	6 (40.0)	8 (53.3)
25°C	16L–8D	0 (0)	3 (15.0)	17 (85.0)	0 (0)	0 (0)	10 (100)	4 (40.0)	5 (50.0)	1 (10.0)
	12L–12D	19 (100)	0 (0)	0 (0)	16 (94.1)	0 (0)	1 (5.9)	17 (100)	0 (0)	0 (0)
20°C	16L–8D	0 (0)	7 (87.5)	1 (12.5)	4 (33.3)	4 (33.3)	4 (33.3)	10 (83.3)	1 (8.3)	1 (8.3)
	12L–12D	9 (100)	0 (0)	0 (0)	11 (100)	0 (0)	0 (0)	11 (100)	0 (0)	0 (0)

Table 5. Mean (±SD) supercooling points of larvae, pupae and adults of *J. orithya orithya*

Condition		Larva	*N*	Pupa	*N*	Adult	*N*
25°C	16L– 8D	–11.5 ± 0.78	10	–16.9 ± 0.36	10	–19.0 ± 0.34	6
20°C	12L–12D	–12.8 ± 0.61	10	–17.7 ± 0.28	10	–19.3 ± 0.45	7
		N.S.		N.S.		N.S.	

N.S.: not significant at 5 % level by *t*-test

Irrespective of temperature and photoperiodic conditions, no female had mature eggs at adult eclosion; even at 3, 7 and 14 days after emergence, no mature females were observed under short day at 20°C. At 25°C, however, mature females were observed as early as 3 days after emergence (Table 3). All the females maintained under long-day conditions at 20 and 25°C had mature eggs seven days after emergence.

Adults exhibited seasonal color polyphenism in response to differences in the experimental photoperiod and temperature treatments (Fig. 3). At 25 and 20°C, all the individuals of both sexes had at least one eyespot on the hind wing and 69.2–100% of them had two eyespots under long-day conditions, whereas most individuals of both sexes had no eyespots under short-day conditions (Table 4). Under long day at 20°C, although most males (88%) had only a single eyespot, females had 0 (33%), 1 (33%) or 2 (33%) eyespots. Although three wing color patterns were observed in females, the proportion of the 'blue' morph increased under short-day or low temperature conditions; the 'orange' morph was prevalent under short day at 30°C (53%), but under long day at 25°C (10%) and 20°C (8%), it accounted for 10% and 8% only, respectively.

Although we did not perform a quantitative analysis, there appeared to be a trend in which the apices of the forewing near the end of vein M1 were more prominent in adults maintained under short day.

The supercooling points for the larvae, pupae and adults were approximately –12, –17 and –20°C respectively (Table 5). Although there was no significant difference between the long- and short-day experimental treatments, the average SCPs of each stage was slightly lower in individuals maintained under short-day conditions.

Discussion

Although the larval and pupal stages of *J. o. orithya* were slightly delayed under short day at 20°C, these stages were not arrested at any of the temperatures or photoperiods tested, suggesting that neither of these stages enter diapause under these conditions. As in Fukuda *et al.* (1983), at 20 and 25°C, the larval stage consisted of either 5 or 6 instars, while at 30°C, it consisted of 5 instars. Although the

Table 6. Summary of differences in wing patterns of *J. orithya orithya* under different photoperiodic and temperature conditions in this study

Temperature	Phenotype	Photoperiod	
		Long day	Short day
High	No. of eyespots[1)]	2	0
	Blue part[2)]	none or small	large
	Orange part[2)]	large	none
Low	No. of eyespots	0–2	0
	Blue part	large	large
	Orange part	none or small	none

1) No. of eyespots on the ventral hindwing in both sexes
2) coloration of the dorsal hind wing in females

proportion undergoing 6 instar larval stages was highest in the 25°C/12L–12D treatment, the effect of photoperiod was not apparent. According to Kiritani (1997), the average t_0 and K values for Japanese lepidopteran species is 10.4°C (N=83) and 463.3 (N=80), respectively. Although the t_0 and K of the egg stage were not determined in this study, the t_0 value of this species is relatively high compared with other lepidopteran species.

As in Nakanishi *et al.* (1975), ovarian development in the adult butterflies examined in this study was clearly delayed by short day/low temperature (20°C) conditions; under long day at 20°C, two females (100%) were confirmed to be mature 7 days after emergence under long day at 20°C. In addition, the proportion of mature females was lower under short day (17%) than under long day (100%) at 25°C, even one week after emergence. These findings suggest that the females of this species enter reproductive diapause when maintained under short day, especially when the larval or pupal stages were exposed to low temperature conditions.

Differences in wing patterns under different photoperiods and temperatures in this study are summarized in Table 6. Under long day/high temperatures, the eyespots on the ventral surface of the hind wings in both sexes, as well as the orange pattern on the dorsal surface of the hind wing, were well developed in females (long-day form). Conversely, the eye spots were obscure and the dorsal surface of the hind wing was usually blue (short-day form) in females maintained under conditions of low temperature and short day in this study. Nakanishi *et al.* (1975) also reported the seasonal color polyphenism in adults of this species under different photoperiods at 25°C, and color-pattern changes induced by exposure to specific temperatures or chemicals have also been reported (Otaki *et al.*, 2005; Otaki, 2008; Kusaba and Otaki, 2009; Mahdi *et al.*, 2010). It is considered that the long-day and short-day forms observed in this study correspond to wet-season and dry-season forms (Nakanishi *et al.*, 1975), high-temperature and low-temperature forms (Tsukada, 1985; Nobayashi, 2002) and summer and autumn forms (Fukuda, 2004), respectively. In a congeneric species, *Junonia almana*, the apices of the forewing become more prominent under short day in Japan (Seki, 1968). Though faint and difficult to quantify, a similar trend was observed in the adults examined in this study. Although Nakanishi *et al.* (1975) examined only the effect of photoperiod, we found that the temperature also affected polyphenism of wing color, particularly in females. In a related species, *Precis octavia*, the temperature is the only environmental factor affecting the incidence of seasonal forms in Africa (McLeod, 1968), while in *Junonia almana* in Japan, the photoperiod has been demonstrated to be more important (Seki, 1967, 1968). From the results of this study, we conclude that the photoperiod is the primary cue inducing polyphenism, but that temperature is also involved.

The cold-hardiness of numerous insects has been reported to increase in response to cool temperatures during the overwintering stage (Baust and Rojas, 1985; Asahina, 1991). For example, Troyer *et al.* (1996) reported that the mean SCP of migrating monarch butterflies, *Danaus plexippus*, was significantly lower than that of non-migrants. Teshirogi (1990) suggested that the cold hardiness of *J. o. orithya* was highest in the adult stage. In the present study, of the three stages examined, the SCP of *J. o. orithya* was lowest in the adult stage and highest in the larval stage, but there was no significant difference between different photoperiods.

Uesugi (1998) reported that the distribution of this species was previously restricted to Yaeyama Island in southwestern Japan, but that they have regularly been observed to overwinter in Okinawa Island from the 1990s onward. Nakagami (1998) also observed this species in Kagoshima in 1987, 1991 and 1996; however, it failed to overwinter there. On the other hand, Fukuda (2004) reared larvae of this species in natural temperature and photoperiodic conditions in Kagoshima City from October 2002 to May

2003 and found that some adults emerged from April to May 2003. According to Fukuda (2008), this species has succeeded in overwintering in Tokunoshima and Okinoerabu Islands, Kagoshima Prefecture in recent years. One of the host plants, *J. procumbens*, is very common along roadsides throughout Honshu, Shikoku and Kyushu in Japan (Osada, 1984), and it is possible that *J. o. orithya* breeds opportunistically in these areas. Kamitani and Yata (2002) estimated that the distribution of a congeneric species, *J. almana*, would expand extensively around the Pacific Ocean if the average temperature increased by 1°C. From the results of this study, it is considered that adults with reproductive diapause are suited to overwinter. Thus the minimum temperature in winter and the condition of food plants in early spring might be important factors for determining the northern distribution limit of this species.

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摘　要

アオタテハモドキ(鱗翅目，タテハチョウ科)の発育と季節多型および耐寒性(平井規央・谷川哲朗・石井実)

アオタテハモドキ *Junonia orithya orithya* を実験室内の25，20°Cの12L–12D（短日）と16L–8D（長日）および30°Cの長日条件下で飼育し，発育，季節型，耐寒性を調査した．幼虫と蛹の平均発育期間は温度の上昇とともに短くなり，幼虫期の発育零点 t_0 と有効積算温度 K は，13.7°Cと208.3日度，蛹期は13.4°Cと99日度と算出された．25°Cでは長日・短日ともに3日目に，20°C長日では7日目に成熟卵を持つ個体が見られたが，20°C短日では14日目にも成熟個体は見られなかった．成虫期には日長と温度による季節型が見られた．雌雄後翅裏面の眼状紋と雌の後翅表面の橙色は高温長日で発達し(長日型)，低温短日では眼状紋が消失して雌の後翅表面は青色となる(短日型)傾向が強く認められた．幼虫(4齢)，蛹，成虫の過冷却点を測定したところ，それぞれ約−12，−17，−20°Cとなり，成虫で最も低かったが，飼育日長による差は認められなかった．

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